Protocols and Upcoming Round Robins: Practical Mechanisms for Reducing Uncertainties

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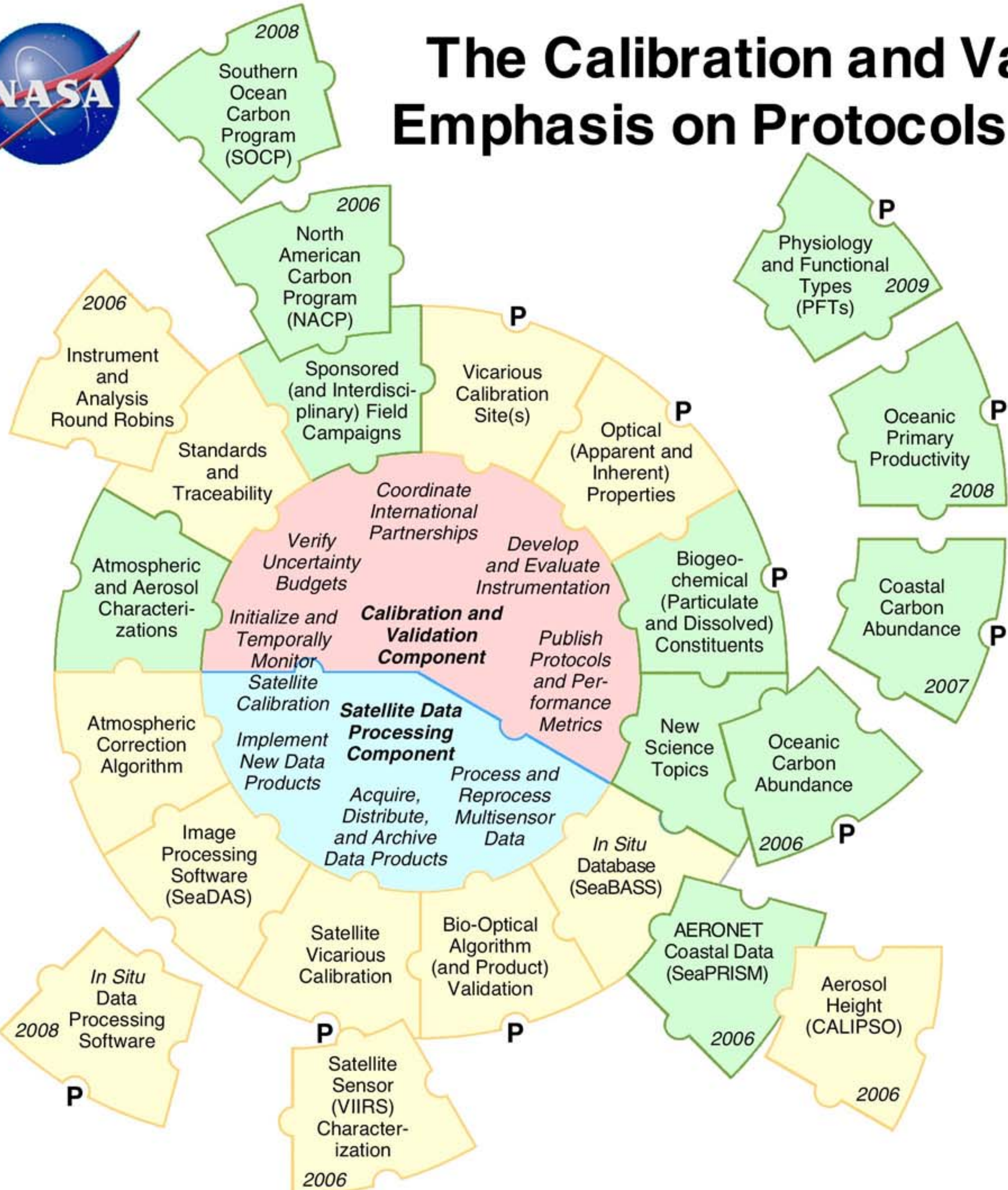
The Calibration and Validation Plan: An Emphasis on Protocols and Uncertainties



In addition to incorporating the review comments into a new version of the Bio-geochemistry Calibration and Validation Plan, the core personnel (blue and red) have met to discuss how best to produce a follow-on implementation plan. As part of the latter, principal and secondary representatives for all of the elements have been determined. A special em-phasis was placed on ensuring the connecting-core (green and yellow) elements had core scien-tists with an established expertise, so an updated *Ocean Optics Protocols* can be produced.

The Premise and Utility of Round-Robins

The premise of a round robin is *all participants use a validated method, which are equally capable of estimating a true result for each "sample," and each sample is analyzed no differently than any other normally analyzed by the method.*

The result from each method is expected to be close to the truth (which is frequently unknown), and the dispersion of the results will be equally expressed above and below the true value. A validated method has no inherent biases, because if one existed it would have been removed by the validation process. The computation of the accuracy (or uncertainty) for each method is based on computing the difference of each result from the truth (usually the average of all data) for each product.

Accuracy estimates how close the result is to the true value while *precision* is an estimate of how exactly the result is determined independently of any true value.

Accuracy is telling a story truthfully, and precision is how similarly the story is repeated over and over again.

Examples of round-robin inquiries for ocean color include the SeaWiFS Intercalibration Round-Robin Experiment (SIRREX), which investigated optical calibrations, and the SeaWiFS Data Analysis Round Robin (DARR), which looked at data products from measurements of the apparent optical properties (AOPs) of seawater.

Highlights from Ocean Color Round Robins

In the progression from the 1st to the 3rd SIRREX, uncertainties in the traceability to NIST for intercomparisons of spectral lamp irradiance and sphere radiance improved from 7–8% to 1–2%.

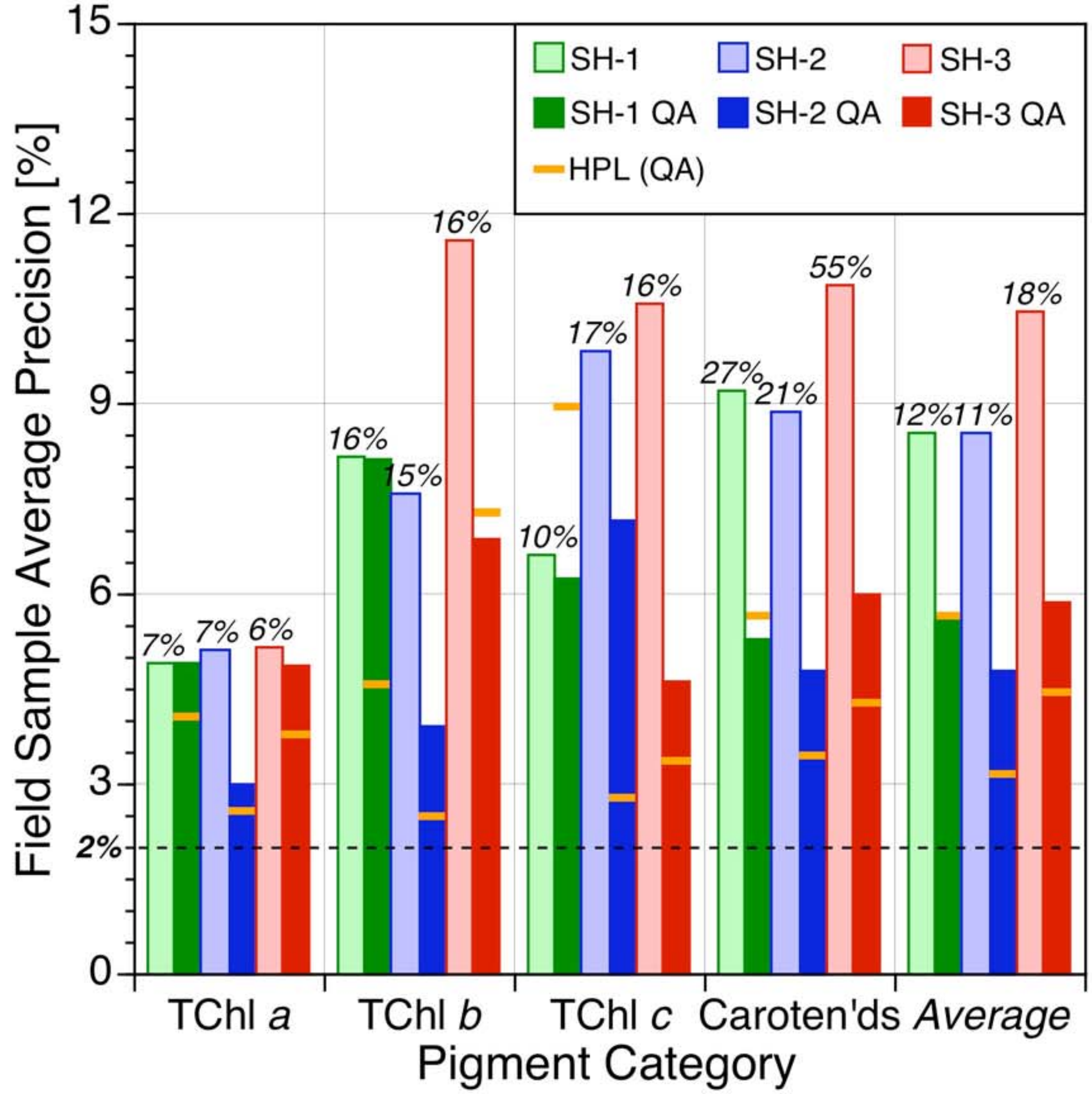
The 4th through 7th SIRREX activities further investigated laboratory and field protocols, and showed calibrations at an uncertainty level of about 2–3% were routinely achievable if the *Ocean Optics Protocols* were carefully implemented. More recently, SIRREX-8 revealed the immersion factors supplied by a commercial manufacturer were more than 10% in error at some wavelengths.

DARR-94 showed differences in methods for determining in-water primary optical parameters were about 3–4%. DARR-00 showed agreement to within 2–3%, and if the processing options were made as similar as possible, agreement to within less than 1% was routinely possible for two of the processors. Much higher uncertainties (greater than 20–50%) were documented, however, and many of these were associated with data products critical to calibration and validation.

Optical parameters do not account for all of the validation requirements. The proper determination of the total chlorophyll *a* concentration (TChl *a*) is central to the objectives of all ocean color missions. More recently, the SeaHARRE activity was initiated to investigate uncertainties in the HPLC quantitation of marine pigments.

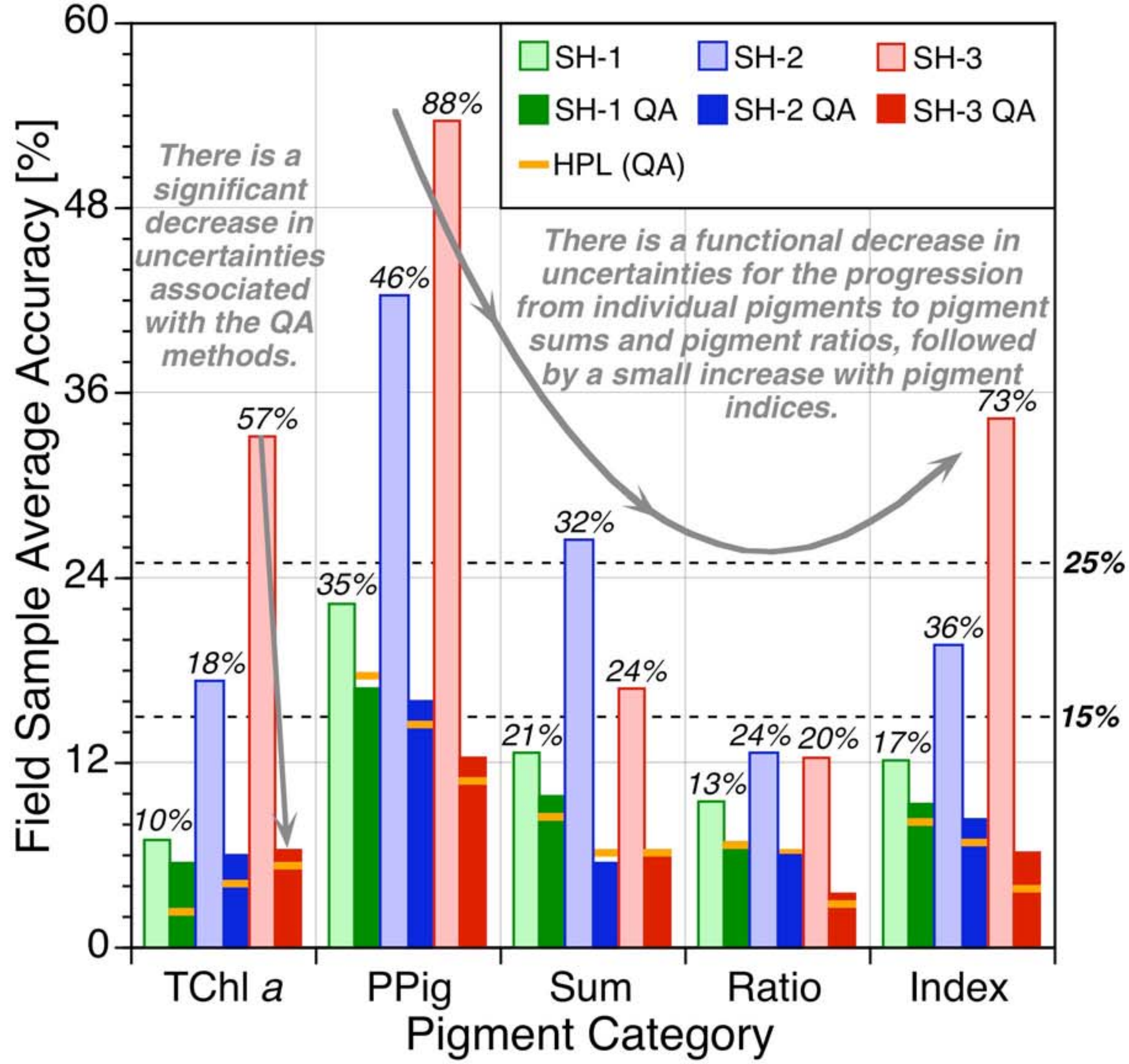
Field Sample Individual (Primary) Pigment Precision (For TChl *a* Spanning 0.020–26.185 mg m^{-3})

The precision of the different methods for all three SeaHARRE intercomparisons are rather similar as was the variability in sample homogeneity (about 2%) arising from the data collection protocol used in the field. The intra- and inter-experimental differences are primarily partitioned between the pigment categories and those methods that were properly validated or *quality assured* (dark bars) and those that were not (light bars). For the latter, the worst-case average result is shown above the bar (individual samples can be much worse).



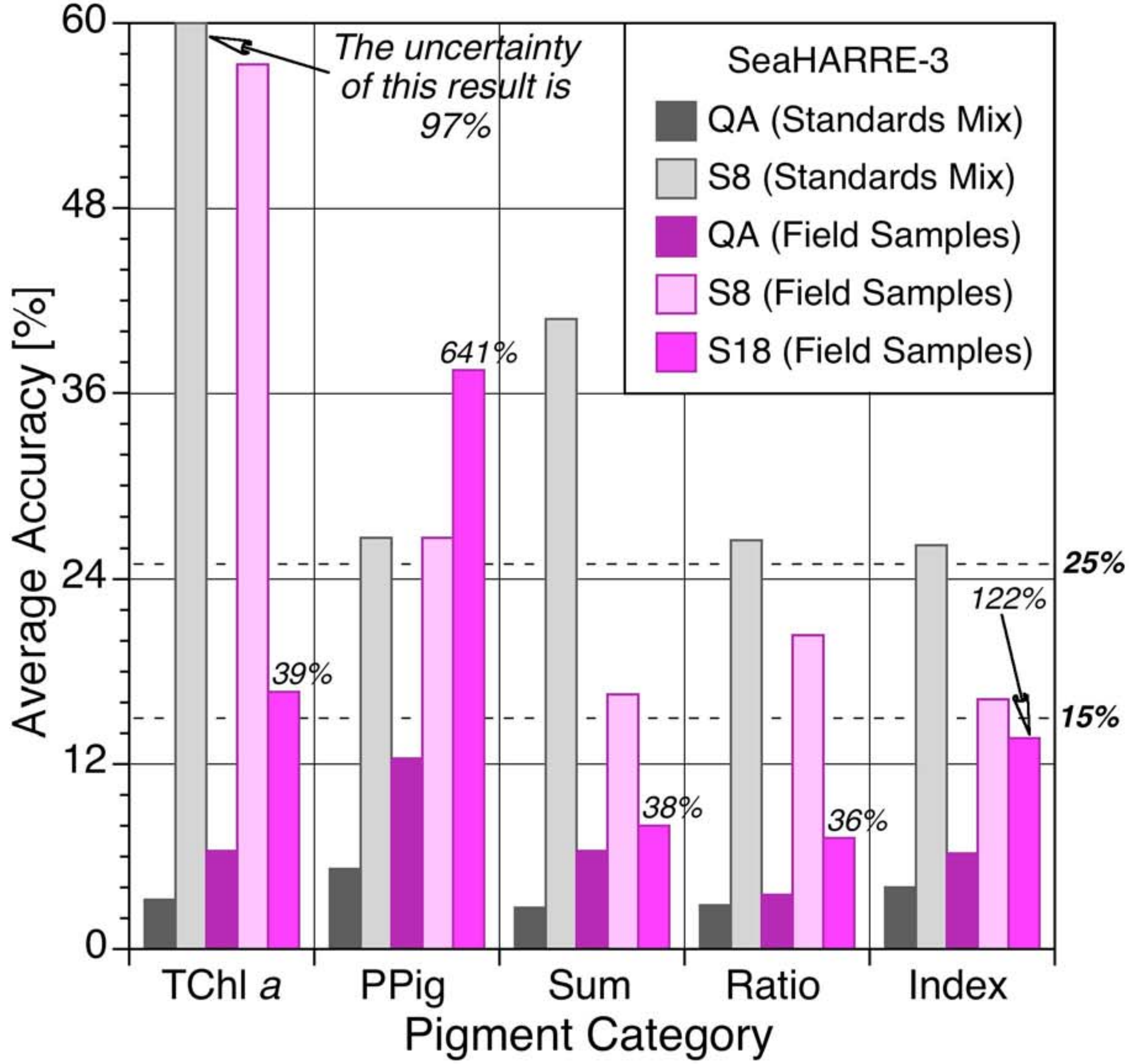
Field Sample Higher-Order Pigment Accuracy

The accuracy of the methods are primarily distinguished by the pigment categories and whether or not the methods were properly quality assured (dark bars) or not (light bars). For the latter, the worst-case average result is shown at the top of the bar (individual samples can be worse). The QA methods have the lowest uncertainties; they always meet the 25% validation requirement and almost always satisfy the 15% refinement objective. Furthermore, there is a functional decrease in the uncertainties for the progression from the primary pigments to the sums and ratios, followed by a small increase with the indices.



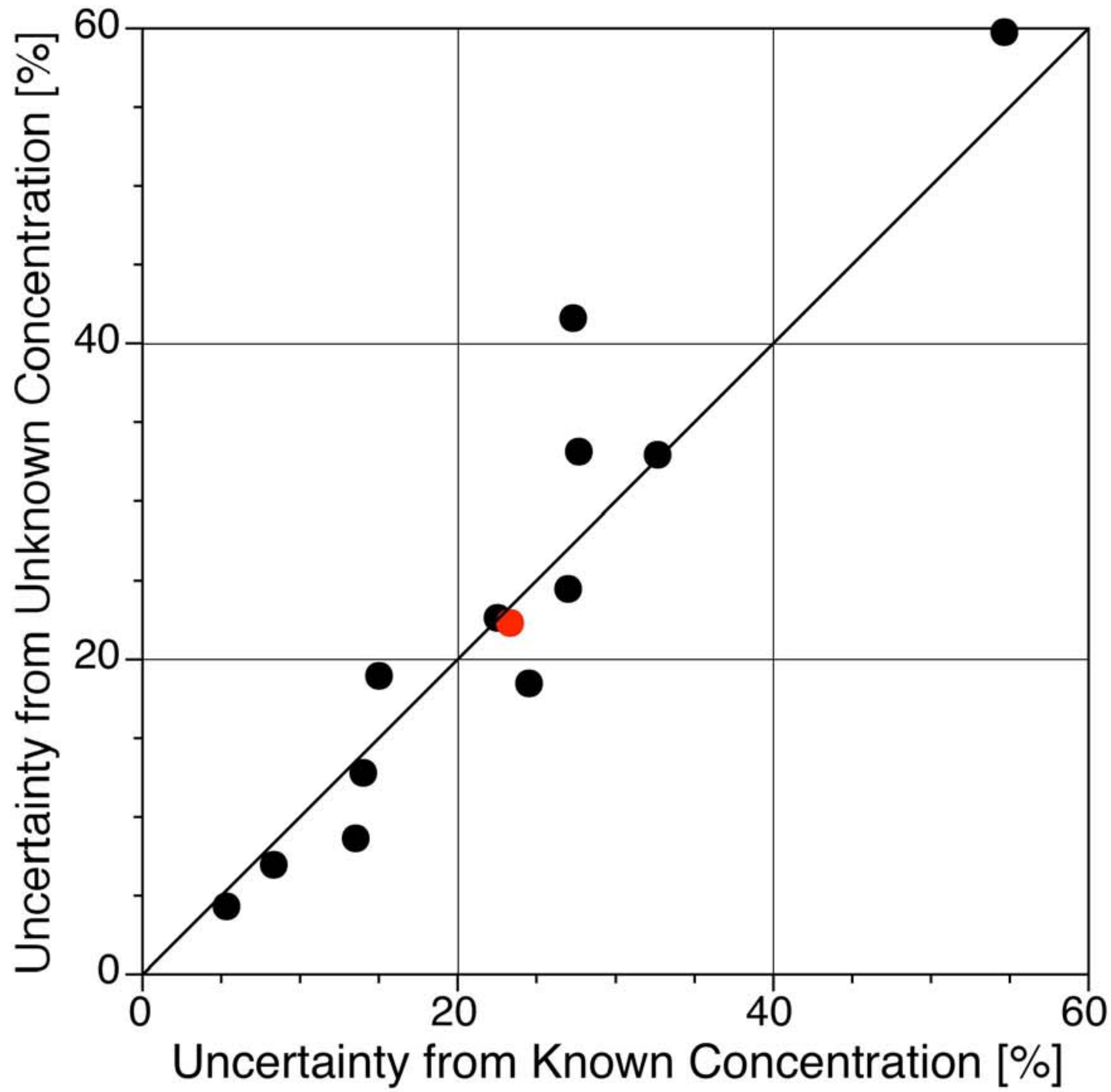
A Summary of the CHORS Results in SeaHARRE-3 (For TChl *a* Spanning 0.02–1.37 mg m-3)

The SeaHARRE-3 results are divided into methods that were properly validated or *quality assured* (dark bars) and those that were not (light bars). For the latter, the worst-case average result is shown above the bar (individual samples can be much worse). CHORS executed two methods based on a C8 and a C18 column, denoted S8 and S18, and both have significant deficiencies: the new S8 method has poor TChl *a* results and nearly adequate PPig results, while the old S18 method has adequate TChl *a* results but very poor PPig results. The higher-order data products are not as notably degraded.



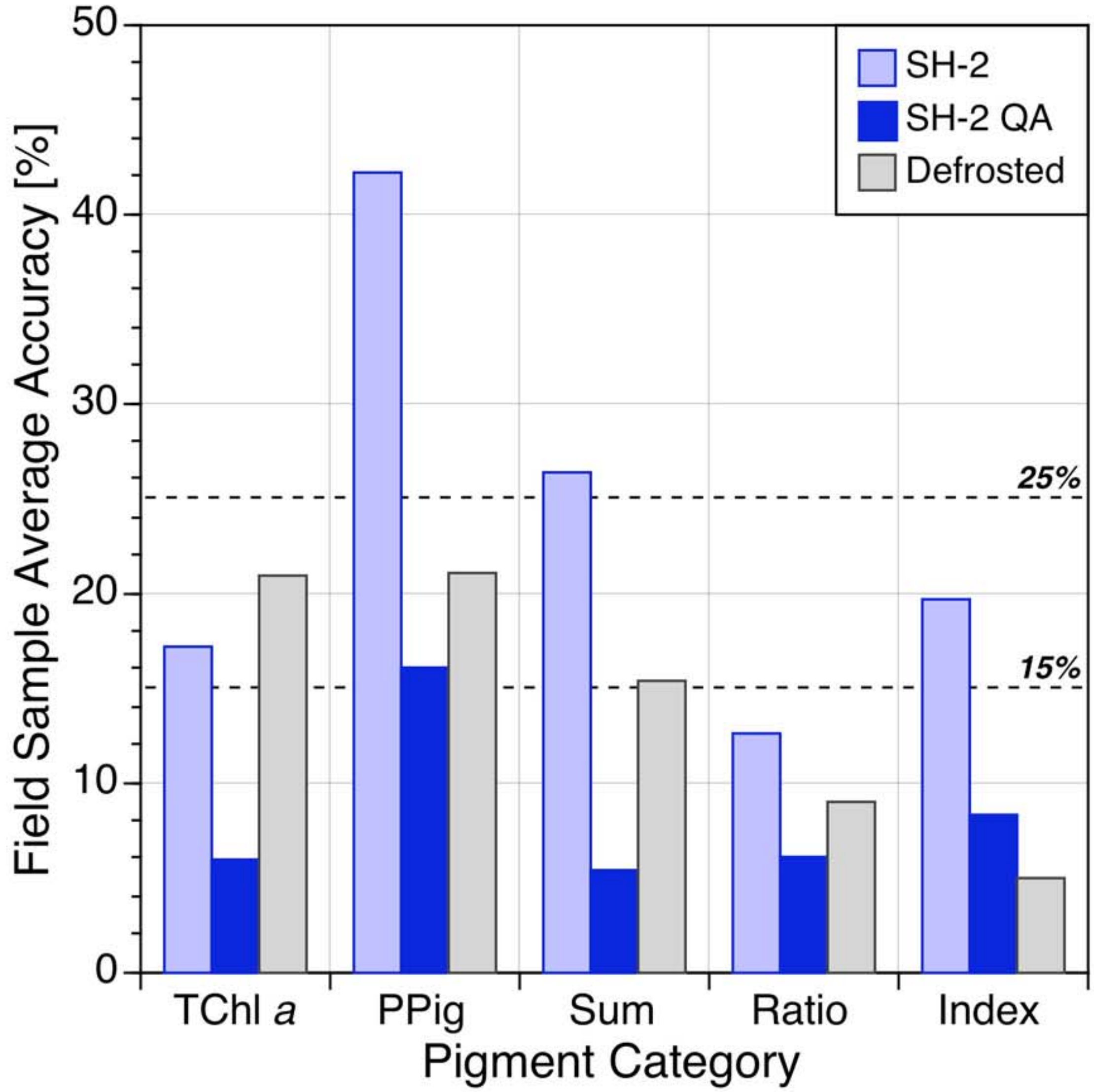
Validation of Approach from Mixed Standards

The validity of the referencing system used to compute accuracy (and, thus, uncertainties) can be investigated using mixed standards (a single solution containing a variety of standards all mixed together in known concentrations). The validation occurs by comparing the uncertainties in the pigment concentrations from the various methods computed using a) the known concentrations within the mix, and b) the average pigment concentrations derived from all the methods. The average uncertainties from these two approaches (the red dot in the figure) agree to within 1.5%.



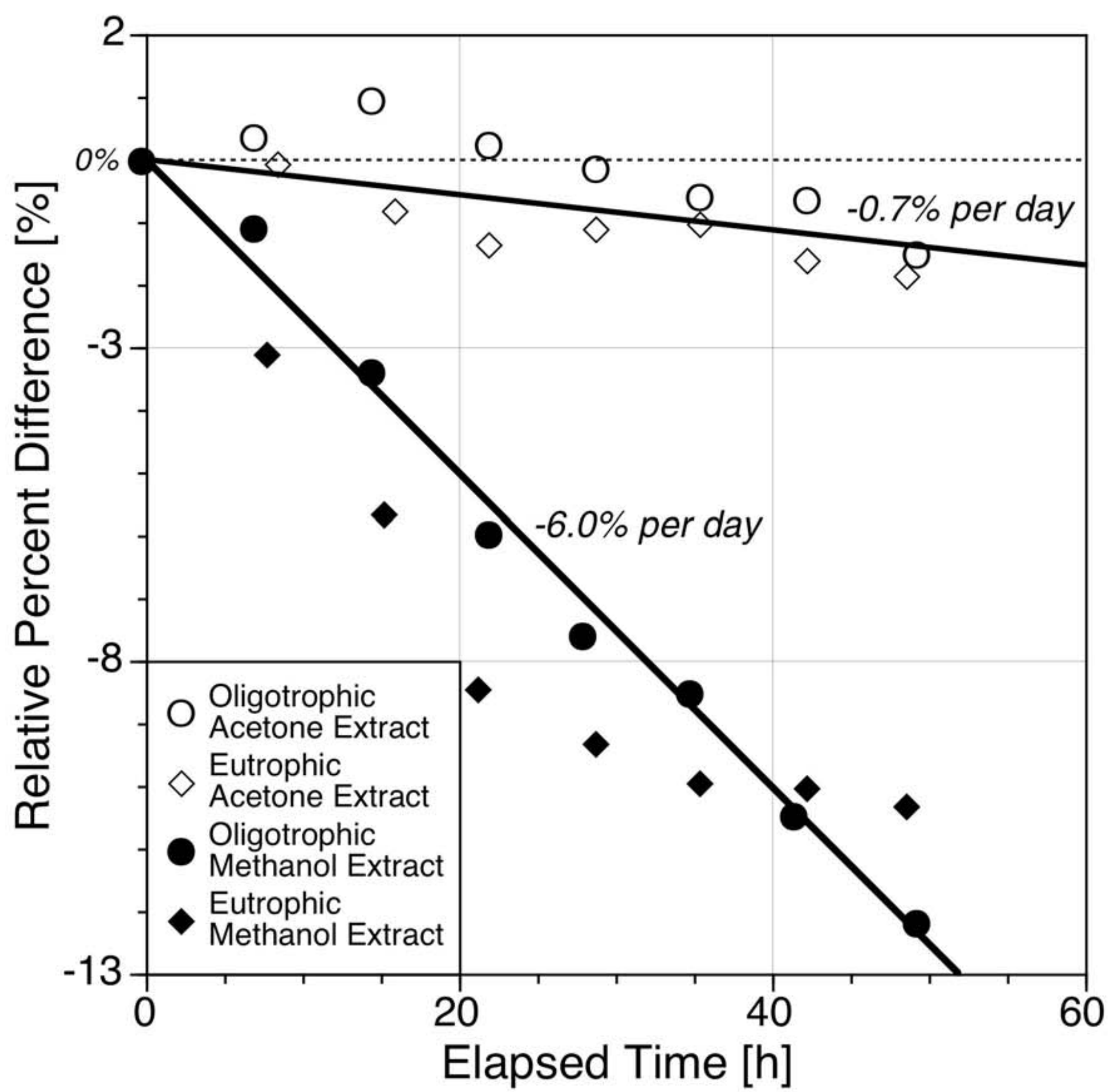
Primary Source of Uncertainty

A recurring presumption in intercomparison experiments is *"Improper handling and storage of the field samples will overwhelm the uncertainty budget,"* that is, the variability from sample decay is much larger than the variability in the methods. This issue was addressed in SeaHARRE-2 by having one of the QA laboratories analyze a set of replicates unequivocally defrosted during shipping. The results showed a quality-assured laboratory analyzing bad samples was superior to a method lacking a QA scheme and analyzing good samples. This was confirmed by the precision data.



Nonetheless, Sample Handling is Important

The previous result showing how a validated (QA) laboratory analyzing bad samples was superior to an unvalidated laboratory analyzing good samples does not mean sample handling is not an important part of minimizing uncertainties. The change in concentration of replicate samples as a function of the elapsed time in the TCAS compartment was measured for acetone and methanol extractions. The quantitation of the PPig pigments for both the oligotrophic and eutrophic samples, as determined by [TChl *a*], degraded steadily, but the methanol extracts were about an order of magnitude more sensitive.



Field Sampling for SeaHARRE-4

The emphasis for SeaHARRE-4 is on coastal (Case-2) waters. The sample set includes 12 different locations from the fjords, estuaries, and bays of Denmark. All samples were collected in triplicate and will be distributed in November.

The sampling plan included a concerted effort to obtain the widest range in water properties possible (8 – 28 PSU) plus a diversity of phytoplankton populations and sizes (including blooms dominated by a single species) to ensure the most complex mix of pigments possible. *At some level, no one area is sufficient, but at another level, any one area is typical as long as the range in complexity of the coastal environment is captured.*

SeaHARRE-4 Participants and Analysis

The laboratories represented in SeaHARRE-4 are a mixture of established and new HPLC practitioners as well as established and new round-robin participants. The new additions have well-established expertise in coastal sampling. Every effort was made to increase the diversity of international groups (e.g., a concerted effort was made to include a South American institute) and methods (e.g., the Zapata method), but the timing of the activity was not necessarily advantageous to the invitees. All of the participants agreed to make an additional analysis with the HPLC extracts to ensure a more comprehensive use of the samples.

Sample Set	*Institute or Laboratory*	*Principal Investigator*	*Country*	*Lab. Code*	*HPLC Pigs*	*Fluor. Chla*	*Spec. Chla*	*Absorp-tion*	*Method*
1	CSIRO	L. Clementson	Australia	C	H		S	A	Van Heukelem and Thomas
2	DHI	L. Schlüter	Denmark	D	H	F	S		Van Heukelem and Thomas
3	GSFC/UMBC	M. Russ	USA	G	H	F	S		Van Heukelem and Thomas
4	HPL	L. Van Heukelem	USA	H	H	F	S	A	Van Heukelem and Thomas
5	JRC	J-F. Berthon	Italy	J	H		S		Van Heukelem and Thomas
6	JRC			J'				A	Van Heukelem and Thomas
7	LOV	H. Claustre	France	L	H		S	A	Van Heukelem and Thomas
8	LOV			L'	H	F	S		Van Heukelem and Thomas
9	USC	J. Pinckney	USA	U	H	F	S		Pinckney
10	FIO	D. Millie	USA	F	H	F	S		Millie
11	SDSU/CHORS	C. Trees	USA	S	H	F			Wright et al.
12	Dalhousie Univ.	C. Normandeau	Canada	N	H	F			Wright et al.
12	*10*	*10*	*6*	*12*	*11*	*8*	*9*	*4*	*4*